Ⅲ. 研究成果の刊行に関する一覧表

雑　誌

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Yamada Y, Ando F, Niino N, Shimokata H	Association of polymorphisms of the androgen receptor and klotho genes with bone mineral density in Japanese women	J Mol Med	83	50-57	2005
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Ⅳ. 研究成果の刊行物・別刷

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ORIGINAL ARTICLE

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Association of polymorphisms of the androgen receptor and klotho genes with bone mineral density in Japanese women

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Abstract Genetic variants of the androgen receptor and klotho protein may contribute to variation in bone mass as well as to predisposition to osteoporosis. The relationship of a CAG repeat polymorphism of the androgen receptor gene (*AR*) and of a −395G→A polymorphism of the klotho gene (*KL*) to bone mineral density (BMD) in Japanese women was examined in a population-based study. The subjects (1,101 and 1,110 women for *AR* and *KL* polymorphisms, respectively) were aged 40–79 years and were randomly recruited to a population-based prospective cohort study of aging and age-related diseases. BMD for the total body, lumbar spine, right femoral neck, right trochanter, and right Ward's triangle was measured by dual-energy X-ray absorptiometry. Genotypes for the *AR* and *KL* polymorphisms were determined by polymerase chain reaction based assays. The number of CAG repeats of *AR* was inversely correlated with BMD for the lumbar spine in premenopausal women but not in postmenopausal women. The $(CAG)_{n\leq 22}$ and $(CAG)_{n\geq 23}$ alleles were designated *S* and *L*, respectively. Among premenopausal women, BMD for the total body was significantly lower in subjects with the *LL* genotype than in those with the *SS* genotype or those in the combined group of *SS* and *SL* genotypes. In contrast, BMD was not associated with *AR* genotype in postmenopausal women. Among all women, BMD for the lumbar spine was significantly lower in subjects with the *GG* genotype of the −395G→A polymorphism of *KL* than in those with the *AA* genotype. BMD was not associated with −395G→A genotype among premenopausal women. In postmenopausal women, BMD for the total body or lumbar spine tended to be lower in subjects with the *GG* genotype than in those with the *AA* genotype or those in the combined group of *GA* and *AA* genotypes. These results suggest that *AR* is a susceptibility gene for reduced BMD in premenopausal Japanese women, and that *KL* is a susceptibility gene for reduced BMD in all women.

Keywords Bone density · Androgen receptor · Klotho protein · Genetics · Osteoporosis

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Abbreviations *AR:* Androgen receptor · *BMD:* Bone mineral density · *PCR:* Polymerase chain reaction

Introduction

Osteoporosis, a major health problem of the elderly, is characterized by a reduction in bone mineral density (BMD) and a deterioration in the microarchitecture of bone, both of which result in predisposition to fractures [1]. Although reproductive, nutritional, and life-style factors influence BMD, family and twin studies have suggested that this parameter is largely heritable and under the control of multiple genes [2, 3, 4]. Genetic linkage analyses [5, 6, 7] and candidate gene association studies [8, 9, 10] have thus implicated several loci and candidate genes in the regulation of bone mass and the prevalence of osteoporosis or osteoporotic fractures. Such candidate genes include those for the androgen receptor (*AR*) and klotho [11, 12].

Androgens play important roles in the development and metabolism of bone [13]. The *AR* is expressed in human osteoblastic cells as well as in human osteoclasts, suggesting that androgens exert direct effects on bone cells [14]. The gene encoding the AR (AR), which is located on human chromosome Xq11-q12, is thus an important candidate susceptibility gene for osteoporosis. Variation in the size of the microsatellite region in the first exon of *AR* is attributable to a CAG repeat polymorphism that encodes a polyglutamine tract comprising 9–35 residues in the amino-terminal domain of the receptor protein [15, 16]. In vitro transfection assays have demonstrated that *AR* proteins with shorter polyglutamine tracts possess greater transactivation activity [17, 18, 19] whereas tract size does not affect the binding of androgens to the receptor [20]. Although the CAG repeat polymorphism of *AR* was shown to be associated with BMD in women or in men in some studies [11, 21, 22, 23, 24], other studies have failed to detect an effect of this polymorphism on BMD or fracture risk [25, 26]. Furthermore, racial differences in the number of CAG repeats have been demonstrated, with African-Americans exhibiting a higher prevalence of short CAG repeat sequences than other ethnic groups [15, 27]. Given the ethnic differences in CAG repeat length as well as in other genetic or environmental influences on BMD, it is important to examine the relationship of the CAG repeat polymorphism of *AR* to BMD in each ethnic group.

Klotho is a type I membrane protein that shares sequence similarity with members of the glycosidase family [28]. Mice deficient in this protein exhibit multiple aging phenotypes and age-related disorders, including a shortened life span, reduced spontaneous activity, arteriosclerosis, infertility, skin atrophy, premature thymic involution, pulmonary emphysema, and osteopenia, although the function of klotho remains to be determined [28]. The osteopenia observed in klotho-deficient mice is accompanied by a reduced turnover of bone; a decrease in bone formation exceeds a decrease in bone resorption, resulting in substantial bone loss that resembles that in aging humans [29]. A human homolog of the mouse klotho gene has been isolated and its structure determined [30]. The human gene (*KL*) comprises five exons and spans approx. 50 kb on chromosome 13q12. Ogata et al. [31] examined the relationship of a CA repeat polymorphism downstream of *KL* to BMD and showed that the alleles corresponding to 22 and 24 repeats are associated with low and high BMD, respectively. Kawano et al. [12] identified eight and six polymorphisms of *KL* in white and Japanese women, respectively, and showed that the –395G→A polymorphism in the promoter of *KL* is associated with BMD in postmenopausal (≥65 years) women of each ethnicity. The sizes of the populations in which this association was detected were only small (55 white, 215 Japanese), however. Large-scale population-based studies are thus required to assess the effect of this polymorphism on BMD.

We attempted to identify genes significantly associated with BMD in Japanese women in a population-based study. *AR* and *KL* are both candidates for genes that confer susceptibility to osteoporosis. We thus examined the relationship of polymorphisms of these genes to BMD in the present study, although there is no apparent biological link between the two genes. Our aim was to identify a single polymorphism significantly associated with BMD for each gene. Among several polymorphisms previously identified in *KL*, only the –395G→A polymorphism has been shown to potentially affect gene function. We therefore selected this polymorphism for our analysis. We have now examined whether the CAG repeat polymorphism of *AR* or the –395G→A polymorphism of *KL* is associated with BMD in Japanese women in a population-based study.

Methods

Study population

The National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA) is a population-based prospective cohort study of aging and age-related diseases [32]. The present study represents a cross-sectional analysis within the NILS-LSA. The subjects of the NILS-LSA are stratified by both age and gender and were randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan [32, 33]. The life-style of residents of this area is typical of that of individuals in most regions of Japan. The NILS-LSA aimed to recruit equal numbers of men and women. Age at the baseline was 40–79 years, and the numbers of participants in each age decade (40s, 50s, 60s, and 70s) were similar. The planned number of participants was 2,400, that is, approx. 300 men and 300 women in each age decade. A total of 7,855 men and women was randomly selected from the community-dwelling population; of these selected individuals 16 were already deceased and 49 had moved away. The remaining 7,790 individuals were invited to attend an explanatory meeting by mail; a total of 3,434 replied, 881 of whom declined to attend the meeting, 2,553 agreed to attend, and 2,513 actually did attend. After the explanatory meeting, 2,267 individuals participated in the initial examination. Thus of the 7,790 individuals contacted by mail and the 34,34 individuals who replied, 29.1% and 66.0%, respectively, enrolled in the study. The subjects will be followed up every

2 years. All participants are subjected at a special center to a detailed examination, which includes not only medical evaluation but also assessment of exercise physiology, body composition, nutrition, and psychology. Among the 2,267 participants 1,128 are women. Eighteen women who had disorders known to cause abnormalities of bone metabolism, including diabetes mellitus, renal diseases, rheumatoid arthritis, and thyroid, parathyroid, and other endocrine diseases, or who had taken drugs such as estrogen, progesterone, glucocorticoids, and bisphosphonates were excluded from the present study. Nine women whose *AR* genotype was not successfully determined were also excluded from the analysis of the relationship of the *AR* polymorphism to BMD.

We examined the relationship of BMD at various sites to the CAG repeat polymorphism of *AR* and to the −395G→A polymorphism of *KL* in 1,101 and 1,110 women, respectively. The study protocol complies with the Declaration of Helsinki and was approved by the Committee on Ethics of Human Research of National Chubu Hospital and the NILS. Written informed consent was obtained from each subject.

Measurement of BMD

BMD for the total body, lumbar spine (L2–L4), right femoral neck, right trochanter, and right Ward's triangle was measured by dual-energy X-ray absorptiometry (QDR 4500; Hologic, Bedford, Mass., USA). The coefficients of variance of the machine were 0.9% (total body), 0.9% (L2–L4), 1.3% (femoral neck), 1.0% (trochanter), and 2.5% (Ward's triangle).

Determination of genotypes

The polymorphic region in exon 1 of *AR* was amplified by the polymerase chain reaction (PCR) with a sense primer labeled at the 5′ end with 6-carboxyfluorescein (5′-ACCTCCCGGCGCCAGTTTG-3′) and with an antisense primer (5′-CTGCTGCTGCCTGGGGCTAG-3′). The reaction mixture (25 μl) contained 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgSO_4$, and 0.4 U KODplus DNA polymerase (Toyobo, Osaka, Japan) in polymerase buffer. The amplification protocol comprised initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s and annealing-extension at 68°C for 30 s; and a final extension at 68°C for 2 min. The size of microsatellite-containing DNA fragments amplified by PCR was determined with a Prism 3100 DNA sequencer with GeneScan and Genotyper software (Applied Biosystems, Foster City, Calif., USA).

Genotypes for *KL* were determined with a fluorescence-based allele-specific DNA primer assay system [34]. The polymorphic region of *KL* was amplified by PCR with allele-specific sense primers labeled at the 5′ end with either fluorescein isothiocyanate (5′-GGCGCCGACCAACTTXCC-3′) or Texas red (5′-GGCGCCGACCAACTTXTC-3′) and with an antisense primer labeled at the 5′ end with biotin (5′-CTAGGGCCCGGCAGGATC-3′). The reaction mixture (25 μl) contained 20 ng DNA, 5 pmol of each primer, 0.2 mmol/l of each deoxynucleoside triphosphate, 2.5 mmol/l $MgCl_2$, and 1 U of rTaq DNA polymerase (Toyobo) in polymerase buffer. The amplification protocol comprised initial denaturation at 95°C for 5 min; 40 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 30 s, and extension at 68°C for 30 s; and a final extension at 68°C for 2 min. The amplified DNA was incubated in a solution containing streptavidin-conjugated magnetic beads in the wells of a 96-well plate at room temperature. The plate was then placed on a magnetic stand, and the supernatants from each well were transferred to the wells of a 96-well plate containing 0.01 mol/l NaOH and were measured for fluorescence with a microplate reader (Fluoroscan Ascent; Dainippon Pharmaceutical, Osaka, Japan) at excitation and emission wavelengths of 485 and 538 nm, respectively, for fluorescein isothiocyanate and of 584 and 612 nm, respectively, for Texas red.

Statistical analysis

Since quantitative data were not necessarily all distributed normally, they were compared by both parametric and nonparametric tests. Comparisons between two groups were performed with the unpaired Student's *t* test or the Mann-Whitney *U* test, and those among three or more groups were compared by one-way analysis of variance and the Tukey-Kramer post hoc test or by the Kruskal-Wallis test (SAS, SAS Institute, Cary, N.C., USA). Since the results obtained with parametric and nonparametric tests were similar, statistical analyses with the former are shown in Tables 1, 2, 3, and 4. BMD values were analyzed with adjustment for age, height, and body weight by the least squares method in a general linear model. Allele frequencies were estimated by the gene-counting method, and the χ^2 test was used to identify significant departure from Hardy-Weinberg equilibrium. The effects of the CAG repeat genotype of *AR*, the −395 G→A genotype of *KL*, or both genotypes on BMD at various sites for all women were evaluated by regression analysis; R^2 and *P* values were calculated from analysis of *AR* genotype and/or *KL* genotype. We considered a *P* value of 0.005 or less to be statistically significant for the multiple comparisons of genotypes with BMD. For other background data, a *P* value of 0.05 or less was considered statistically significant. We also calculated the statistical power to detect differences in BMD among women with different genotypes, where α=0.0167 among three groups, α=0.0083 among four groups, and β=0.1.

Results

The distribution of the number of CAG repeats in *AR* for all women ranged from 12 to 37 (22.8±2.9; Fig. 1). The number of CAG repeats was significantly related to L2–L4 BMD for premenopausal women, but not for postmenopausal or total women (Fig. 2). Among premenopausal women BMD for the lumbar spine decreased as the number of CAG repeats increased. Since the mean number of CAG repeats was 22.8, we designated $(CAG)_{n\leq 22}$ and $(CAG)_{n\geq 23}$ alleles as short (*S*) and long (*L*) alleles, respectively.

The distributions of *SS*, *SL*, and *LL* genotypes of *AR* were in Hardy-Weinberg equilibrium, and age, height,

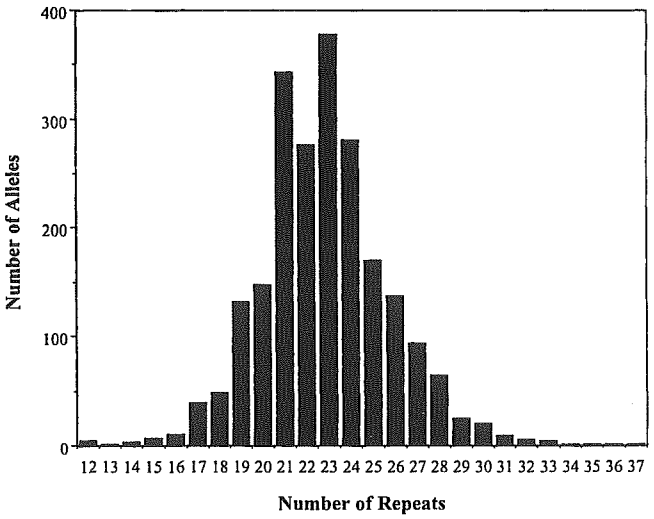

Fig. 1 Distribution of the number of CAG repeats in *AR* in 1,101 women (2,202 alleles)

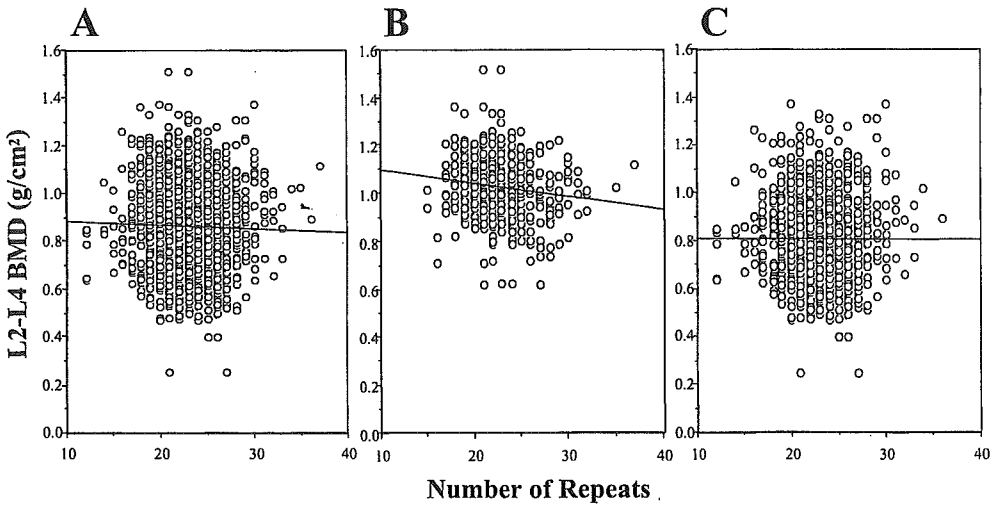

Fig. 2 Relationship between the number of CAG repeats in *AR* and L2-L4 BMD. **A** All women (n=1,101, 2,202 alleles); r=–0.01967, P=0.3584. **B** Premenopausal women (n=275, 550 alleles); r=–0.14455, P=0.0007. **C** Postmenopausal women (n=809, 1,618 alleles); r=0.00751, P=0.7644

Table 1 BMD and other characteristics of all women (n=1,101) according to the CAG repeat genotype of *AR*. BMD values are adjusted for age, height, and body weight

	SS (n=238, 21.6%)	*SL* (n=535, 48.6%)	*LL* (n=328, 29.8%)	*SS* + *SL* (n=773, 70.2%)	*SL* + *LL* (n=863, 78.4%)
Age (years)	58.9±0.7	59.1±0.5	59.9±0.6	59.1±0.4	59.4±0.4
Height (cm)	151.8±0.4	151.2±0.3	151.0±0.3	151.4±0.2	151.1±0.2
Body weight (kg)	52.3±0.5	52.4±0.4	52.6±0.5	52.4±0.3	52.5±0.3
BMD (g/cm^2)					
Total body	0.972±0.006	0.965±0.004	0.961±0.005	0.967±0.003	0.963±0.003
L2–L4	0.884±0.008	0.861±0.005*	0.860±0.007	0.868±0.005	0.860±0.004**
Femoral neck	0.686±0.006	0.677±0.004	0.675±0.005	0.680±0.003	0.676±0.003
Trochanter	0.576±0.005	0.570±0.004	0.568±0.005	0.572±0.003	0.569±0.003
Ward's triangle	0.514±0.008	0.506±0.005	0.505±0.006	0.508±0.004	0.506±0.004

* $P \leq 0.05$, ** $P \leq 0.01$ vs. *SS* (statistical power to detect differences in BMD among women with *SS*, *SL*, or *LL* genotypes is 0.1% of the largest value)

and body weight did not differ among genotypes, for all women (Table 1). BMD for the lumbar spine with adjustment for age, height, and body weight tended to be lower in the combined group of women with the *SL* or *LL* genotypes or in women with the *SL* genotype than in those with the *SS* genotype; the *P* values for these differences, however, did not achieve statistical significance.

To examine the possible influence of menopause on the relationship between genotype and BMD, we analyzed BMD and other characteristics for premenopausal and postmenopausal women independently. Because of their small number (n=17) perimenopausal women were excluded from the analysis. The distributions of *SS*, *SL*, and *LL* genotypes of *AR* were in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for premenopausal or postmenopausal women (Table 2). For premenopausal women, BMD for the total body was significantly ($P \leq 0.005$) lower in those with the *LL* genotype than in those with the *SS* genotype or those in the combined group of *SS* and *SL* genotypes. The difference in BMD for the total body between the *SS* genotype and the *LL* genotype was 3.9% (expressed as a proportion of the larger value). In contrast, BMD was not associated with *AR* genotype in postmenopausal women.

The distribution of –395G→A genotypes of *KL* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes for all women (Table 3). BMD for the lumbar spine was significantly ($P \leq 0.005$) lower in women with the *GG* genotype than in those with the *AA* genotype; the difference in L2–L4 BMD between these two groups (expressed as a percentage of the larger value) was 7.9%.

We also analyzed the relationship of BMD and other characteristics to *KL* genotype for premenopausal and postmenopausal women independently (Table 4). The distributions of –395G→A genotypes of *KL* were in Hardy-Weinberg equilibrium, and age and body weight did not differ among genotypes in premenopausal or postmenopausal women. Height did not differ among *KL* genotypes in premenopausal women, but postmenopausal women with the *GG* genotype were taller than were those with the *GA* genotype or those in the combined group of *GA* and *AA* genotypes. In premenopausal women, BMD was not associated with –395G→A genotype. In postmenopausal women, although there was a trend ($P \leq 0.05$) for BMD for the total body or lumbar spine to be lower in subjects with the *GG* genotype than in those with the *AA* genotype or those in the combined group of *GA* and *AA*

Table 2 BMD and other characteristics of women (n=1,084) according to menopausal status and the CAG repeat genotype of *AR*. BMD values are adjusted for age, height, and body weight

	Premenopausal women (n=275)				Postmenopausal women (n=809)			
	SS (n=62, 22.6%)	*SL* (n=134, 48.7%)	*LL* (n=79, 28.7%)	*SS* + *SL* (n=196, 71.3%)	*SS* (n=173, 21.4%)	*SL* (n=393, 48.6%)	*LL* (n=243, 30.0%)	*SS* + *SL* (n=566, 70.0%)
Age (years)	46.2±0.6	46.0±0.4	46.6±0.5	46.0±0.3	63.6±0.7	63.8±0.4	64.4±0.6	63.7±0.4
Height (cm)	154.4±0.6	154.4±0.4	154.5±0.5	154.4±0.3	150.8±0.5	150.0±0.3	149.8±0.4	150.3±0.3
Body weight (kg)	53.9±1.0	54.4±0.7	54.6±0.9	54.2±0.6	51.7±0.6	51.7±0.4	51.8±0.5	51.7±0.3
BMD (g/cm^2)								
Total body	1.111±0.010	1.102±0.007*	1.068±0.009**· *****	1.105±0.006	0.922±0.007	0.916±0.004	0.921±0.006	0.918±0.004
L2–L4	1.050±0.014	1.031±0.010	0.997±0.013****	1.037±0.008	0.826±0.010	0.801±0.006	0.809±0.008	0.809±0.005
Femoral neck	0.780±0.011	0.777±0.008	0.762±0.010	0.778±0.006	0.654±0.006	0.640±0.004	0.643±0.005	0.645±0.004
Trochanter	0.668±0.010	0.664±0.007	0.642±0.009***	0.665±0.006	0.544±0.006	0.537±0.004	0.541±0.005	0.539±0.003
Ward's triangle	0.674±0.015	0.666±0.010	0.641±0.013	0.668±0.008	0.457±0.009	0.449±0.006	0.456±0.007	0.452±0.005

*$P\leq0.01$, **$P\leq0.005$ vs. *SS*, ***$P\leq0.05$, [4]*§$P\leq0.01$, [5]*$P\leq0.001$ vs. *SS* + *SL* (statistical power to detect differences in BMD among premenopausal or postmenopausal women with *SS*, *SL*, or *LL* genotypes is 0.2% or 0.1% of the largest value, respectively)

Table 3 BMD and other characteristics in all women (n=1,110) according to the −395G→A genotype of *KL*. BMD values are adjusted for age, height, and body weight

	GG (n=812, 73.2%)	*GA* (n=268, 24.1%)	*AA* (n=30, 2.7%)	*GA* + *AA* (n=298, 26.8%)
Age (years)	59.4±0.4	58.9±0.7	58.8±2.0	58.9±0.6
Height (cm)	151.5±0.2	150.7±0.4	151.0±1.1	150.7±0.4
Body weight (kg)	52.5±0.3	52.1±0.5	53.2±1.5	52.2±0.5
BMD (g/cm^2)				
Total body	0.962±0.003	0.970±0.005	0.994±0.016	0.973±0.005
L2–L4	0.860±0.004	0.872±0.008	0.934±0.023**·***	0.878±0.007*
Femoral neck	0.678±0.003	0.675±0.005	0.692±0.016	0.677±0.005
Trochanter	0.569±0.003	0.572±0.005	0.601±0.015	0.575±0.005
Ward's triangle	0.504±0.004	0.511±0.007	0.537±0.021	0.513±0.007

* $P\leq0.05$, **$P\leq0.005$ vs. *GG*, ***$P\leq0.05$ vs. *GA* (statistical power to detect differences in BMD among women with *GG*, *GA*, or *AA* genotypes is 0.1% of the largest value)

Table 4 BMD and other characteristics in women (n=1093) according to menopausal status and the −395G→A genotype of *KL*. BMD values are adjusted for age, height, and body weight.

	Premenopausal women (n=278)				Postmenopausal women (n=815)			
	GG (n=199, 71.6%)	*GA* (n=71, 25.5%)	*AA* (n=8, 2.9%)	*GA* + *AA* (n=79, 28.4%)	*GG* (n=602, 73.9%)	*GA* (n=191, 23.4%)	*AA* (n=22, 2.7%)	*GA* + *AA* (n=213, 26.1%)
Age (years)	46.3±0.3	46.0±0.5	45.5±1.6	45.9±0.5	63.9±0.3	64.0±0.6	63.6±1.8	63.9±0.6
Height (cm)	154.4±0.3	154.7±0.6	152.9±1.7	154.5±0.5	150.5±0.2	149.1±0.4*	150.4±1.3	149.2±0.4**
Body weight (kg)	54.4±0.6	53.8±1.0	55.0±2.9	53.9±0.9	51.9±0.3	51.4±0.6	52.5±1.7	51.5±0.6
BMD (g/cm^2)								
Total body	1.094±0.006	1.087±0.010	1.133±0.029	1.092±0.009	0.914±0.004	0.928±0.006	0.946±0.018	0.930±0.006*
L2–L4	1.023±0.008	1.023±0.013	1.110±0.040	1.032±0.013	0.803±0.005	0.818±0.009	0.874±0.027*	0.824±0.009*
Femoral neck	0.774±0.006	0.765±0.011	0.781±0.032	0.767±0.010	0.643±0.003	0.643±0.006	0.662±0.018	0.645±0.006
Trochanter	0.661±0.006	0.646±0.010	0.684±0.029	0.650±0.009	0.536±0.003	0.547±0.006	0.572±0.017	0.549±0.006
Ward's triangle	0.656±0.008	0.658±0.014	0.714±0.042	0.664±0.013	0.450±0.005	0.458±0.008	0.475±0.025	0.459±0.008

* $P\leq0.05$, **$P\leq0.01$ vs. *GG* (statistical power to detect differences in BMD among premenopausal or postmenopausal women with *GG*, *GA*, or *AA* genotypes is 0.2% or 0.1% of the largest value, respectively)

Table 5 Effects of the CAG repeat genotype of *AR*, the −395G→A genotype of *KL*, or both genotypes on BMD in all women (n=1,110). The R^2 and P values were derived from regression analysis of *AR* genotype (0=*SS*, 1=*SL*=*LL*) and/or KL genotype (0=*GG*=*GA*, 1=*AA*)

	AR genotype		*KL* genotype		*AR* and *KL* genotypes	
	R^2	P	R^2	P	R^2	P
Total body						
AR	0.0023	0.1255	0.0015	0.2151	0.0026	0.1016
KL					0.0015	0.2157
L2–L4						
AR	0.0045	0.0307	0.0045	0.0287	0.0048	0.0256
KL					0.0046	0.0281
Femoral neck						
AR	0.0031	0.0735	0.0008	0.3457	0.0034	0.0621
KL					0.0008	0.3464
Trochanter						
AR	0.0013	0.2399	0.0027	0.0921	0.0016	0.1991
KL					0.0027	0.0958
Ward's triangle						
AR	0.0015	0.2124	0.0013	0.2382	0.0017	0.1856
KL					0.0013	0.2432

genotypes, the P values for these relationships did not achieve statistical significance.

Finally, the effects of the CAG repeat genotype of *AR*, the −395G→A genotype of *KL*, or both genotypes on BMD at various sites in all women were evaluated by regression analysis (Table 5). Although there was a trend ($P \leq 0.05$) that *AR* genotype and *KL* genotype affected BMD for the lumbar spine, this difference was not statistically significant. The effects of the two polymorphisms on BMD were statistically independent.

Discussion

The CAG repeat polymorphism of *AR* has previously been shown to be associated with osteoporosis in men. In a study of white men, repeat length was inversely correlated with BMD, with long repeats [$(CAG)_{n>21}$] being associated with lower phalangeal BMD, higher bone turnover, and increased bone loss [21]. A study of Finnish men, however, did not detect an association between this polymorphism of *AR* and BMD [26]. In women overrepresentation of certain *AR* genotypes (combinations of alleles with 22, 23, 24, or 25 repeats) was found among pre- or perimenopausal individuals with low BMD [11]. A Danish study demonstrated a higher frequency of long alleles in women with osteoporotic fractures and a negative correlation between allele size and BMD [22]. In contrast, no association was observed between the *AR* polymorphism and BMD in a study of Finnish women [25]. The effects of the CAG repeat polymorphism of *AR* on BMD have not previously been determined for premenopausal and postmenopausal women independently in the same ethnic group.

We have now shown that the number of CAG repeats in *AR* is inversely correlated with BMD for the lumbar spine in premenopausal Japanese women, and that BMD for the total body is significantly lower in premenopausal women with two $(CAG)_{n\geq 23}$ alleles than in those with one or two $(CAG)_{n\leq 22}$ alleles. Our observation that long repeat alleles are associated with reduced BMD is consistent with the similar previous observation in Danish women [22].

This association between BMD and the CAG repeat polymorphism is possibly attributable to the fact that the transactivation activity of the *AR* is inversely correlated with the number of CAG repeats [17, 18, 19]. In vitro observations thus suggested that a decrease of six CAG repeats results in a 12% increase in ligand-dependent transactivation activity of the *AR* [18]. This relationship between repeat length and transactivation activity is due in part to variation in the basal activity of the *AR* and to functional interaction of the polyglutamine tract with coactivators [35, 36]. In addition, the serum concentration of androgens is related to the CAG repeat polymorphism of *AR*, with short alleles being associated with higher levels of androgens in premenopausal women [37]. This finding supports our observation that the *AR* polymorphism is associated with BMD in premenopausal, but not postmenopausal, women, although the definition of short alleles differed between this previous study [$(CAG)_{n\leq 19}$] [37] and our study [$(CAG)_{n\leq 22}$] and postmenopausal women were not examined in the previous study [37].

The mean number of CAG repeats for the *AR* in our population (22.8) was greater than that previously reported in Danish women (21.9) [24] or in Danish normal (20.5) or osteoporotic (21.0) women [22]. Furthermore, the mean number of CAG repeats in African-American men (20.1) was smaller than that in white men (22.1) or Asian men (22.1) [15]. These differences in repeat number may account at least in part for the differences in BMD or in the prevalence of osteoporosis among ethnic groups. Since the mean number of CAG repeats was 22.8 in our study population, we designated $(CAG)_{n\leq 22}$ and $(CAG)_{n\geq 23}$ alleles as short (*S*) and long (*L*) alleles, respectively. The cutoff value for the CAG repeat number in our study was thus greater than that in previous studies: $(CAG)_{n\leq 21}$ [24], $(CAG)_{n\leq 20}$ [22], $(CAG)_{n\leq 19}$ [37], and $(CAG)_{n\leq 18}$ [25] for the *S* allele.

The somatic cells of most females contain two X chromosomes, only one of which is active. The process of X chromosome inactivation, which occurs early in de-

velopment, is usually random, resulting in the generation of tissues with approximately equal numbers of cells in which the active X chromosome is of maternal or paternal origin [38]. Deviation from such an equal distribution of the two cell types can occur, however. A skewed pattern of X chromosome inactivation affecting the CAG repeat polymorphism of *AR* has been associated with other hormone-related diseases in women [38, 39, 40]. Given that no information is available on the relative extents of inactivation of the *S* and *L* alleles of *AR* in the present study, the evaluation of BMD in individuals with the *SL* genotype requires caution.

The −395G→A and 1818C→T polymorphisms of *KL* have previously been associated with BMD for the total body in white women aged 65 years or older and with that for the distal radius in Japanese women of the same age group, with BMD decreasing according to the rank orders of genotypes *GG* >*GA* >*AA* for the −395G→A polymorphism and *CC* >*CT* >*TT* for the 1818C→T polymorphism [12]. In the present study we examined the relationship of BMD at various sites to the −395G→A polymorphism but not to the 1818C→T polymorphism, since the latter is a synonymous polymorphism (His→His) and appears not to have a functional effect. We found that the −395G→A polymorphism of *KL* is significantly associated with BMD for the lumbar spine in all women, with the *GG* genotype representing a risk factor for reduced BMD. However, when premenopausal and postmenopausal women were analyzed separately, this polymorphism was not significantly related to BMD in either group, although there was a trend for the *GG* genotype to be associated with low BMD in postmenopausal women. The alleles of the −395G→A polymorphism associated with reduced BMD thus differ between the present study (*G* allele) and the previous study (*A* allele) [12]. Although the reason for this discrepancy is unclear, there are two major differences between the two studies: (a) The number of subjects in which the association was detected was greater in our study (n=1,110) than in the previous study (n=55 for white women, n=215 for Japanese women). (b) BMD was compared among *KL* genotypes with adjustment for age, height, and body weight in our study, but BMD was not adjusted in the previous study. However, it is possible that the −395G→A polymorphism of *KL* is in linkage disequilibrium with other polymorphisms of *KL* or of nearby genes that are actually the determinants of BMD. Although we adopted a strict criterion of statistical significance ($P \leq 0.005$) for the association of genotypes with BMD, we cannot completely exclude the possibility of statistical errors such as false positives.

Evidence suggests that the −395G→A polymorphism of *KL* affects promoter function [12]. Electrophoretic mobility-shift analysis revealed that the amount of DNA-protein complex formed by the *G* allele of the promoter was greater than that formed by the *A* allele, suggesting that the binding of one or more proteins to the promoter is impaired by the G→A substitution, which may affect the expression of *KL*. The effect of this polymorphism on the transcriptional activity of *KL*, however, remains to be determined.

There were no subjects with clinical vitamin D deficiency such as osteomalacia in the present population. However, National Nutrition Survey in 2001 suggested that in approximately 25% of Japanese individuals, the amount of vitamin D taken was smaller than that of daily requirement (100 IU). Serum concentrations of free thyroxine in three subjects (0.3%) slightly exceeded the normal range (0.77–1.93 ng/dl). It is thus possible that subclinical vitamin D deficiency or thyrotoxicosis affected the results obtained in the present study.

In conclusion, our present results suggest that *AR* is a determinant of BMD in premenopausal Japanese women, with the $(CAG)_{n\geq 23}$ allele representing a risk factor for reduced BMD. *KL* is also a determinant for bone mass in Japanese women, with the *G* allele being a risk factor for reduced BMD. The effects of both polymorphisms on BMD were statistically independent.

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ORIGINAL ARTICLE

Relationships of muscle strength and power with leisure-time physical activity and adolescent exercise in middle-aged and elderly Japanese women

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Aim: The purpose of the present study is to assess the relationships of muscle strength and power with recent leisure-time physical activity and exercise during adolescence in middle-aged and elderly Japanese women.

Methods: The subjects consisted of 1128 community-dwelling women aged 40–79 years. They were interviewed about their physical activity habits during leisure time in the past 12 months and exercise they engaged in during adolescence. Muscle function was measured as grip strength, knee extension strength and leg extension power. Subjects were grouped into three intensity levels for leisure-time physical activity and as to whether or not they engaged in adolescent exercise. The relationships of muscle strength and power with leisure-time physical activity and adolescent exercise were assessed using analysis of covariance controlled for age, smoking status, annual income and education level.

Results: The proportion of subjects that participated in leisure-time physical activity was 67.1% (light, 33.7%; moderate or heavy, 33.4%). The subjects that engaged in adolescent exercise represented 41.9% of the total. There was a significant relationship between leisure-time physical activity and adolescent exercise. In the analysis of covariance controlled for age, smoking status, annual income and education level, leisure-time physical activity and adolescent exercise had significant main effects on all muscle strength and power measurements. However, there was no interaction effect between leisure-time physical activity and adolescent exercise.

Conclusion: The results suggest that current leisure-time physical activity and adolescent exercise benefit muscle function in middle-aged and elderly women.

Keywords: adolescent exercise, leisure-time physical activity, middle-aged and elderly, muscle power, muscle strength.

Introduction

Regular physical activity and exercise are closely associated with muscle function. Previous cross-sectional studies suggest that regular physical activity, such as leisure-time physical activity or playing sports, is positively

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associated with muscle strength and power,[1,2] and some longitudinal studies showed that elderly women who were very physically active maintained their knee extensor strength at a higher level.[3,4] Intervention studies also documented the effects of strength training on the improvement of muscle strength.[5–11] The stimuli of physical activity or exercise on skeletal muscle may help maintain or improve muscle function.

On the other hand, since muscle function develops rapidly during childhood and adolescence, reaching a peak during adulthood, the beneficial effects of exercise on muscle development seem to be greater during this period. Moreover, Malina noted that tracking of physical activity in youth was associated with physical performance in later life.[12] Therefore, it is important to pay attention not only to current physical activity but also to adolescent physical activity to prevent a decline of muscle strength and power in the elderly. However, little is known about the contribution of both current and adolescent physical activity on muscle function in middle-aged and elderly people.

The purpose of the present study was to assess the relationships of muscle strength and power with current leisure-time physical activity (LTPA) and past adolescent exercise (AEX) in middle-aged and elderly Japanese women. Although the age-associated changes in muscle strength and power were similar by gender, women are generally weaker than men across the adult life span.[13–18] Since women have a longer period of dependency than men, in spite of women's longer life expectancy,[19,20] poor muscle strength and power may be a more serious physical problem for elderly women, resulting in disability or difficulty in performing basic daily tasks. For these reasons, we focused on women in this study.

Methods

Subjects

The data for the present study were derived from baseline data collected as part of the initial survey of the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA). In this project, the normal aging process has been assessed using detailed questionnaires and examinations including clinical evaluations, blood chemistries, anthropometrical measurements, physical fitness tests, nutritional analysis, and psychological tests. Details of the study are reported elsewhere.[21] The initial survey of NILS-LSA involved 2267 men and women aged 40–79 years. They were gender- and decade age-stratified random samples living in Obu city and Higashiura-cho Aichi Prefecture, Japan. Written informed consent was obtained from all the participants. Out of these 2267 participants, 1128 women were used as subjects in this study.

Muscle function

Grip strength (GS): a handgrip dynamometer (Takei Co., Japan) was used to assess grip strength in kilograms. The subjects stood holding a handgrip dynamometer with their hands by their sides while squeezing with maximum force alternating the left and right hands. The average of two readings from each hand was used as the measurement result.

Knee extension strength (KES): the subjects were seated in an adjustable straight-back chair (Takei Co., Japan) with the pelvis, knee and ankle fixed at 90°. A strain gauge was attached to the distal leg by a strap just above the ankle. The subjects tried to extend their legs using maximum isometric force with the knee flexed at 90° while the amplified output from the strain gauge was recorded. The average of the maximum force that each leg attained after three attempts was used as the measurement result in kilograms.

Leg extension power (LEP): leg extension power was measured with the help of a sledge ergometer in a sitting position (Takei Co., Japan). The acceleration of the sledge was 0.73 m/s and the sledge stroke was 0.79 m. The subjects were fastened by a seat belt to the chair. In the starting position, the feet were placed on a footplate attached perpendicularly to a rail, and the knee angle was adjusted to 90°. The subjects were asked to extend their legs as quickly and powerfully as possible, so that the footplate started sliding horizontally on the rail. The highest result of eight attempts was taken as the measurement result in watts.

A medical doctor asked the subjects about their health condition before the muscle function tests. Subjects with serious pains, physical injuries or illness of the orthopedic or cardiovascular systems were excluded. All muscle function tests were performed on the same day.

Physical activity

Leisure-time physical activity (LTPA): trained interviewers using a questionnaire developed by the Japanese Lifestyle Monitoring Study Group asked subjects about the frequency and duration of their physical activity habits during leisure time for the past 12 months.[22] This questionnaire was modified from the Minnesota Leisure-time Physical Activity Questionnaire, one of the most widely used physical activity questionnaires.[23] Activities that were performed at least once a week and for 10 min were defined as LTPA, and classified into three levels: light (approximate physical intensity; 2.5 METs [metabolic equivalents]); moderate (4.5 METs); heavy (> 6.5 METs) (Table 1). Sedentary activities in LTPA, for example, bonsai, were excluded.

Adolescent exercise (AEX): subjects were also interviewed in the same questionnaire about the frequency and duration of their participation in physical exercise

Table 1 The classification of leisure-time physical activity

Level	Approximate intensity (METs)	Description	Examples
Light	2.5	Activity such as walking	Walking, gymnastic exercise, gardening, etc.
Moderate	4.5	Sweating activity that one can do comfortably	Brisk walking, dancing, swimming for pleasure, etc.
Heavy	≥ 6.5	Vigorous exercise with heavy breathing	Several sports activities (swimming, tennis, badminton etc.)

METs, metabolic equivalents.

Table 2 Characteristics of the subjects

	n	Mean ± SD
Age (years)	1128	59.3 ± 10.9
Height (cm)	1128	151.3 ± 6.1
Weight (kg)	1128	52.4 ± 8.2
Body mass index (kg/m^2)	1128	22.9 ± 3.3
Body fat (%)	1120	31.5 ± 5.2
Grip strength (kg)	1106	23.8 ± 5.1
Knee extension strength (kg)	780	25.2 ± 6.8
Leg extension power (w)	1048	301.4 ± 107.1
Smoking status (%; currently)	1126	7.3
Annual income (%; ≥ ¥6 500 000)	1055	54.7
Education level (%; > high school)	1123	23.1

or sports, such as club activities, in addition to compulsory physical exercise at school from 12 to 20 years of age. Activities that were engaged in at least once a week over 1 year were defined as AEX.

Other parameters

Height and weight were measured using a digital scale. Body mass index was calculated by weight divided by height squared (BMI; kg/m^2). Body fat mass was assessed by dual X-ray absorptiometry (DXA; QDR-4500A, Hologic, USA). Lifestyle factors including smoking status, annual income and education level were also determined by questionnaire.

Statistical analysis

The participation rate in physical activity was calculated as the percentage of subjects who reported such activities in a multiple response format. The subjects were divided into three groups according to the intensity of LTPA: no LTPA, LTPA (N); participation in only light activities, LTPA (L); participation in moderate or heavy activities, LTPA (H). Because there were only a few subjects who participated in heavy LTPA, we combined the subjects who engaged in moderate LTPA and heavy LTPA together as LTPA (H). They were also divided into those who engaged in adolescent exercise, AEX (+), and those that did not, AEX (–). The Cochran–Mantel–Haenszel method was used to examine the relationship between LTPA and AEX. The relationship of muscle function with LTPA and AEX was analyzed using the analysis of covariance controlled for age, smoking status, annual income and education level. Statistical testing was performed using the Statistical Analysis System release.8.2 (SAS Institute Inc. NC, USA).[24] Significant probability levels were considered to be less than 0.05.

Table 3 The participation rates in leisure-time physical activity (LTPA) and adolescent exercise (AEX)

	Levels	*n* (%)
LTPA	None	371 (32.9)
	Light	380 (33.7)
	Moderate or heavy	377 (33.4)
AEX		473 (41.9)[†]

[†]Total number of the subjects who participated in AEX.

Results

The characteristics of the subjects are summarized in Table 2. The mean and standard deviation (SD) of age was 59.3 ± 10.9 years. The averages of the anthropometric parameters, height, weight, BMI and percent body fat, were 151.3 ± 6.1 cm, 52.4 ± 8.2 kg, 22.9 ± 3.3 kg/m^2 and 31.5 ± 5.2%, respectively. The averages for muscle strength and power, GS, KES and LEP, were 23.8 ± 5.1 kg, 25.2 ± 6.8 kg and 301.4 ± 107.1 w, respectively. The proportions of people who currently smoked, had an annual income of over 6 500 000 yen, and had an education beyond high school were 7.3, 54.7 and 23.1%, respectively.

Table 3 shows the participation rates in LTPA and AEX. Subjects who did not participated in leisure-time

Table 4 The relationship between leisure-time physical activity (LTPA) and adolescent exercise (AEX)

	LTPA (N)	LTPA (L)	LTPA (H)	*P*-value
AEX (–)	228 (34.8)	242 (37.0)	185 (28.2)	< 0.001
AEX (+)	143 (30.2)	138 (29.2)	192 (40.6)	

Numbers (%) are shown for those who participated in LTPA or AEX.
Cochran-Mantel-Haenszel test, df = 1.

Table 5 Covariance models for muscle functions

	Grip strength		Knee extension strength		Leg extension power	
	df	*F*-value	df	*F*-value	df	*F*-value
LTPA	2	5.6*	2	11.8*	2	13.8*
AEX	1	28.8*	1	17.3*	1	8.6*
LTPA × AEX	2	0.2	2	0.1	2	0.9
Error	1023		718		972	
r^2		0.32		0.23		0.26

*$P < 0.05$.
Covariance models were controlled for age, smoking status, annual income and education level.
LTPA, leisure-time physical activity; AEX, adolescence exercise.

physical activity in the past 12 months accounted for 32.9%. Subjects who participated in light activities and in moderate or heavy activities were 33.7% and 33.4%, respectively. About 42% reported that they had participated in AEX. The most popular sports in adolescence were volleyball, table tennis and softball.

There is a significant difference in the participation rates of LTPA relative to the level of AEX (Table 4). The AEX (+) subjects were more likely to participate in higher levels of LTPA than the AEX (–) subjects ($P < 0.001$).

The relationships of muscle strength and power to both LTPA and AEX are shown in Table 5. As a result of analysis of covariance controlled for age, smoking status, annual income and education level, LTPA and AEX had significant main effects on GS, KES and LEP ($P < 0.05$). However, there was no interaction effect between LTPA and AEX, which indicated the subjects who participated in higher levels of LTPA or the subjects who participated in AEX independently have stronger muscle strength and power than those who did not participate (see Fig. 1).

Discussion

The aim of the present study was to examine the relationships of muscle strength and power with current leisure-time physical activity and past adolescent exercise in middle-aged and elderly women. We found that people who participated in higher levels of current leisure-time physical activity or adolescent exercise had stronger grip strength, knee extension strength and leg extension power than those who did not participate.

Our result that current leisure-time physical activity was associated with positive muscle functions is supported by previous studies. In a cross-sectional study, Hunter *et al.* reported that women who participated in recreational activity or exercise had stronger hand-grips and greater knee extensor strength across age groups (from the 20s through 80s) than those who did not participate in those activities.[25] Van Heuvelen *et al.* also reported that leisure-time physical activity is positively and age-independently associated with grip strength among a community-based sample aged 57 years and older.[1] As for muscle power, it was shown that current physical and sporting activities could contribute to muscular strength and power improvement among healthy subjects over 60 years old.[26] Current leisure-time physical activity may help preserve muscle strength and power. Improvements in muscle function due to training adaptation have been observed in several intervention studies.[7–11] Although strength training may increase muscle function, the results of our study suggest that stimuli from leisure-time physical activity, which does not necessarily include strength training, may also affect the development of muscle function.

Furthermore, although the data were not shown, we also analyzed the data excepted for the subjects who participated in heavy leisure-time physical activity from LTPA (H), and attained results similar to those we have presented here. Accordingly, the physical activity required to maintain or develop muscle function seems to be only moderate activity.

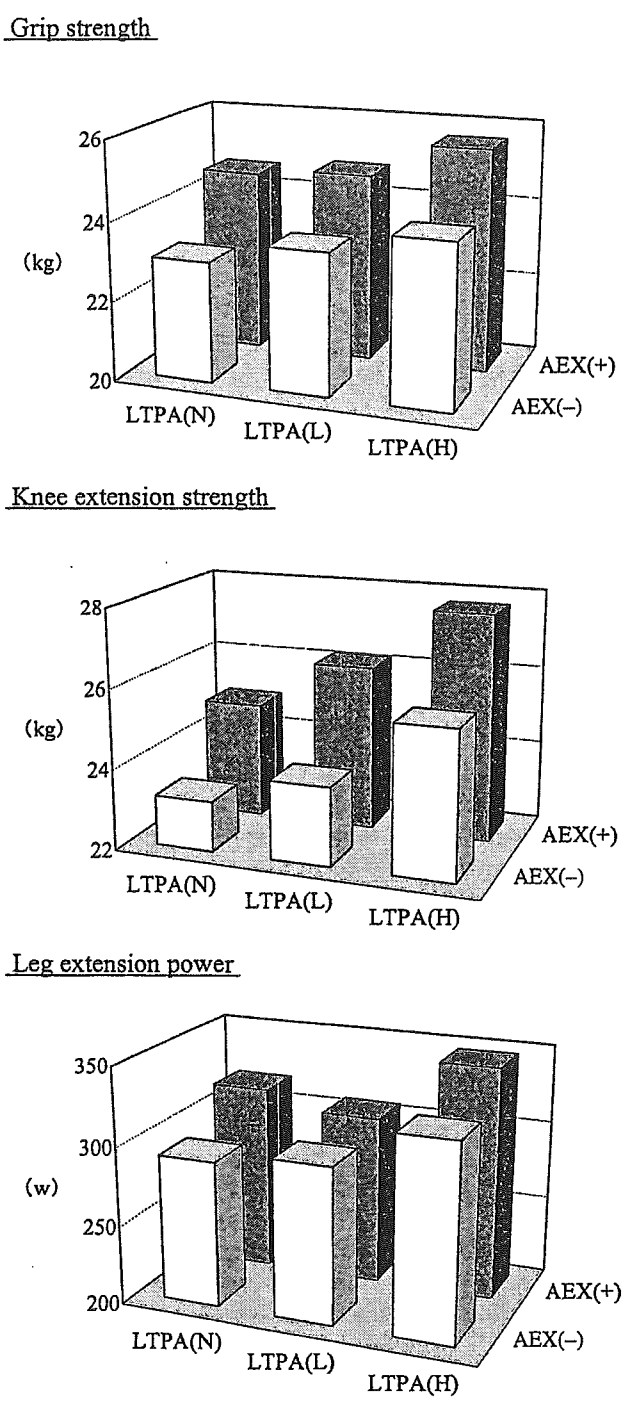

Figure 1 The relationships of leisure-time physical activity and adolescent exercise with grip strength, knee extension strength and leg extension power controlled for age, smoking status, annual income and education level. (AEX(–), without adolescent exercise; AEX(+), with adolescent exercise; LTPA(N), no leisure-time physical activity; LTPA(L), light leisure-time physical activity; LTPA(H), moderate and heavy leisure-time physical activity.)

The most interesting finding in our investigation was that adolescent exercise related positively to muscle function in middle-aged and elderly women.

It seems to contribute to strong muscle strength and power partly because the beneficial training effect of adolescent exercise remains in later life and partly because the continuation of physical activity from adolescence affects muscle function.

It is well known that resistance training increases muscle strength and power.[7–11] Therefore, training during adolescence may influence the build-up of muscle strength and power during the same period, although the positive benefit of training on muscle function decreases during a detraining period.[27–29] Moreover, Connelly *et al.* suggested that long-term detraining effects on muscle function increased in elderly women because of aging and illness,[30] which makes it difficult to conclude that the adolescent training effect on muscle strength or power persisted for more than 20 years. It is unclear whether the effects of exercise or sport participation for longer than one year during adolescence are actually retained until middle and old age. Further studies are needed.

Since it has been suggested that past participation in regular exercise is highly predictive of exercise participation in current exercise in later life,[31,32] which means that people who have participated in regular exercise tend to continue exercising, the continuation of physical activity may be the factor associated with positive muscle function. Hiraoka *et al.* reported that individuals examined at centers for health promotion who maintained the habit of regular exercise from school days to the present had a higher level of physical fitness than those who did not.[33] Although there were only 40 samples, Gauchard *et al.* also showed, that among the elderly aged over 60 years, individuals who participated in regular exercise for more than 40 years had stronger muscle strength and power compared to those who stopped physical activity at least 30 years before.[26] Frändin *et al.* reported that there was no association between activity level during the teenage years and muscle strength at age 76, but they also reported that the activity level throughout life was associated with walking speed among elderly women.[34] These results suggest that the continuation of exercise from early in life is associated with the prevention of declining muscle function in later life. Actually, our data indicated a relationship between current leisure-time physical activity and adolescent exercise as regards participation rates. It is possible that adolescent exercise is a predictor of participation in leisure-time physical activity later in life and the subjects who engaged in adolescent exercise continued exercising afterward.

It may be presumed that physical exercise or sports in youth are essential for the establishment of an exercise habit and the preference for an active lifestyle.

Participation in adolescent exercise may have not only direct effect on muscle functions but also indirect effect through increment of participation in leisure-time physical activity in later life.

There are some limitations in our study. First, because it was a cross-sectional study, cause and effect cannot be distinguished. For example, leisure-time physical activity can increase muscle function, but the level of muscle function may also contribute to participation in leisure-time physical activity. Secondly, the objective reliability of the results may be somewhat limited because the physical activity was based on self-rating, although previous study has already confirmed the reliability of the method,[22] and trained interviewers conducted the questioning in order to maintain the validity of our data.

Finally, the criterion for acceptance of physical activity in the study was lower than the recommended criterion of exercise for improving physical fitness (i.e. two or more times per week). In addition, we assessed only the intensity of physical activity so that the effect of physical activity duration on muscle function could not be identified. Furthermore, it should be remembered that the habit of engaging in physical activity often includes a motivation to participate in a healthy and active lifestyle, which, in itself, might affect muscle function. Nevertheless, the results of this study afford some perspective for preventing the decline of muscle strength and power, and thus, maintaining the quality of life in the elderly.

In conclusion, women who participated in a higher level of leisure-time physical activity or those who participated in adolescent exercise have significantly stronger muscle strength and power than those who did not. These results suggest that current leisure-time physical activity and adolescent exercise are beneficial for maintaining muscle strength and power in middle-aged and elderly women.

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調査・研究

中年期地域住民における転倒の発生状況

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はじめに

高齢者の転倒は発生頻度が高く，種々の要因が複合して起こり，寝たきりの主要な原因といわれる[1)]．転倒は在宅高齢者で20％弱[2-4)]，施設入居高齢者で13～37％[5-7)]と幅広い範囲で発生すると報告されているが，それよりも若い年齢層を対象とした転倒調査の報告はほとんどない．若い年齢層は転倒が少なく問題が小さいという可能性もあるが，実際に若年層や中年層で転倒の頻度が少ないかを検討した研究は少ない．死因における転倒・転落の割合は40代半ばから増加傾向にあるようにみえる[8)]．したがって，40～50代の中年期においても転倒は重要な事故といえるだろう．また高齢者の転倒の特徴を知る上で，それよりも若い世代の転倒の発生状況を知り，その結果と比較検討することは意義があると考えられる．そこで，われわれは40～50代の中年期の地域住民を対象に，転倒をした人の割合，発生時刻，場所などの発生状況に関する調査を行なった．

1. 方　法

1）調査対象

対象は愛知県大府市および東浦町在住の40～59歳の中から年齢・性別層化無作為抽出により選定された人で，国立長寿医療センター研究所疫学研究部の主催する老化に関する長期縦断疫学調査（National Institute for Longevity Sciences-Longitudinal Study of Aging：NILS-LSA）の第一次調査（1997年11月～2000年4月）に参加した1,130名（男性572名，女性558名）である．NILS-LSAは，地域在住の中高齢者を対象に老化の進行について観察を行ない，老化に関する基礎データを蓄積し，老化や老年病の成因を疫学的に解明することを目的とした学際的な縦断的疫学調査である．NILS-LSAの詳細については他論文を参照されたい[9)]．なお，対象者に事前の説明会で，調査の目的・内容について詳しい説明を行ない，文書による同意を得た．本調査については現国立長寿医療センター研究所の倫理委員会にて承認を受けている（承認番号14）．

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